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THE ULTRASTRUCTURE OF THE SKIN OF HUMAN EMBRYOS

I. THE INTRAEPIDERMAL ECCRINE SWEAT DUCT*

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Previous investigators of the ultrastructure of the eccrine sweat duct have limited their studies entirely to specimens obtained from the skin of adult humans and adult lower mammals. No detailed information about the origin and development of the intraepidermal eccrine sweat duct has been available.

MATERIALS AND METHODS

Ten fresh human embryos, 12 to 22 weeks old and ranging in size from 6 to 18 cm (crown-rump length), were obtained. Skin from the sole of the foot was cut into small pieces and was fixed in a solution consisting of 1% osmium tetroxide buffered to pH 7.5 with veronal-acetate and adjusted to physiologic osmolarity by the addition of 4.5% sucrose. The specimens then were dehydrated through a graded series of alcohols and embedded in Araldite. Sections were cut on an LKB Ultrotome and stained with lead citrate (1). Most sections were stained in addition with either 1% phosphotungstic acid in 50% ethanol or with 1% uranyl acetate in distilled water. Sections were examined with an RCA EMU-3G electron microscope.

RESULTS

No evidence of development of eccrine sweat gland anlagen was observed in the embryos younger than 12 weeks old (6 cm long).

In embryos 12–13 weeks old (6–7 cm long), the stratum germinativum consisted of a single layer of darkly staining oval to columnar cells attached to the basement membrane by half-desmosomes and to each other and to the cells of the stratum intermedium by desmosomes. The anlagen of eccrine sweat glands were first seen as regularly spaced undulations of the stratum germinativum (Fig. 1). An intact basement membrane covered each undulation. The cells forming the eccrine sweat gland anlagen were oblong and palisading. They lay more closely together than the other cells in the stratum germinativum. Except for this crowding, the cells of the eccrine sweat gland anlagen did not differ from the other cells of the stratum germinativum. There were distinct intercellular spaces between the interlocking villi of these palisading cells of the anlagen. In these spaces glycogen particles were visible. The cells of the germinative layer outside the sweat gland anlagen also showed intercellular spaces filled with glycogen particles. The glycogen particles lay together in aggregates and stained darkly with lead citrate (2) (Fig. 1). In the stratum intermedium glycogen particles appeared within the cytoplasm, often surrounding the nucleus. The amount of intracytoplasmic glycogen increased in the upper strata including the incompletely keratinized stratum corneum (Fig. 2). Although tonofilaments were abundant in the cytoplasm of the cells forming the eccrine sweat gland anlagen as well as in the adjacent cells of the stratum germinativum, only a few of them had aggregated into tonofibrils. Most of the tonofibrils present were attached to the desmosomes as short tufts. Oval mitochondria were numerous, and the rough-surfaced endoplasmic reticulum was well developed in the cells of the sweat gland anlagen.

In embryos 14–15 weeks old (8–10 cm long), the tips of the eccrine sweat gland anlagen had penetrated deeply into the dermis and had begun to form coils. In the overlying epidermis as well as in the future eccrine sweat duct ridge the columns of cells destined to form the intraepidermal† sweat ducts were recognizable as distinct types of cells. Each column was composed of two distinct cylindrical layers. Each layer, one cell thick (Fig. 3), extending from the basal cell layer was composed of the *inner cells* in which an initial formation of lumen was observed. In the earliest stage of the

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† The term intraepidermal as used in this report includes the eccrine sweat duct ridge.

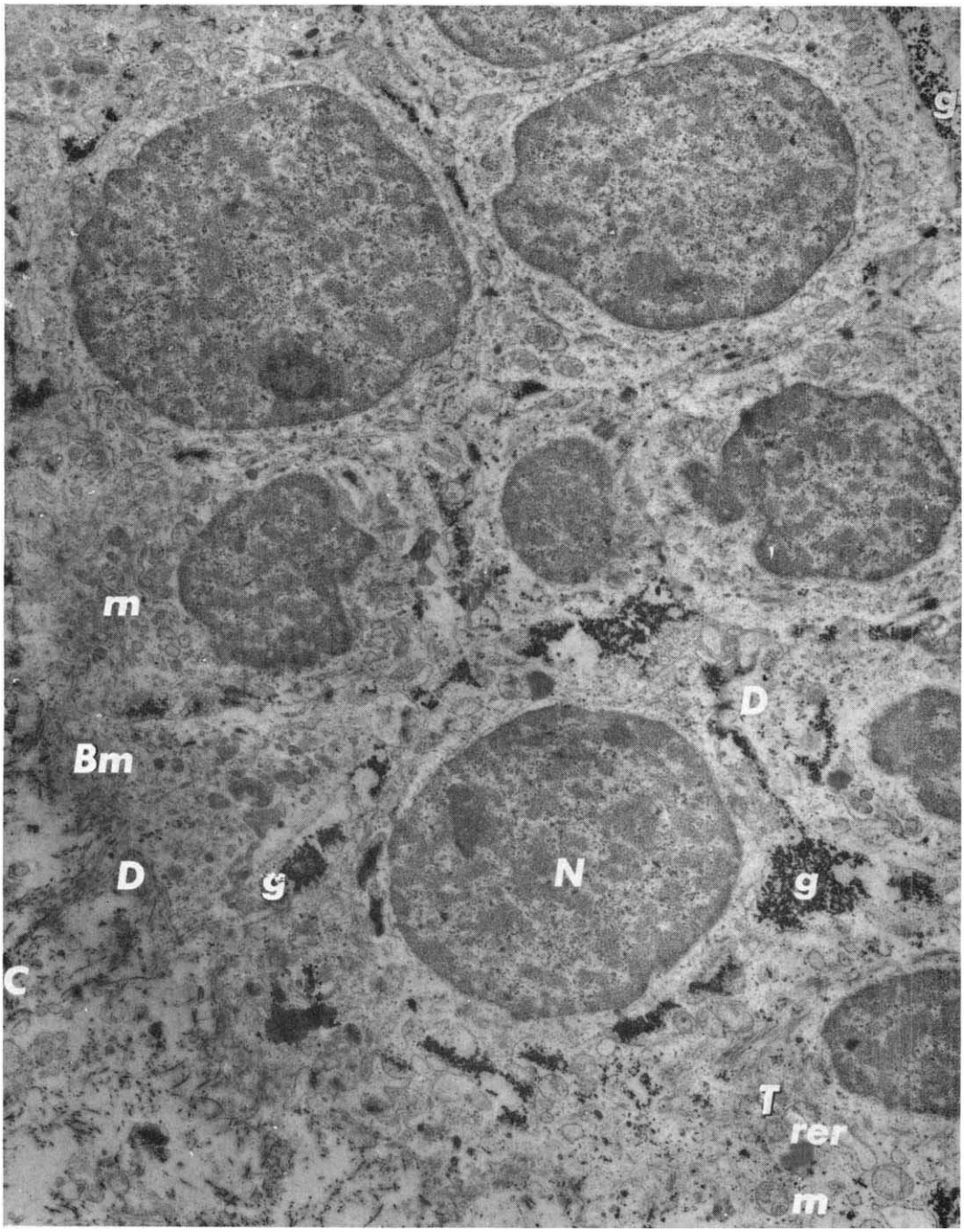

FIG. 1. *Eccrine sweat gland anlage, 13 weeks old embryo.* Glycogen particles (g) are found only in the wide intercellular spaces. Bm: basement membrane. C: collagen. D: desmosomes. m: mitochondria. N: nucleus of germinative cell. rer: rough-surfaced endoplasmic reticulum. T: tonofilaments. Stained with lead citrate. (× 56,150.)

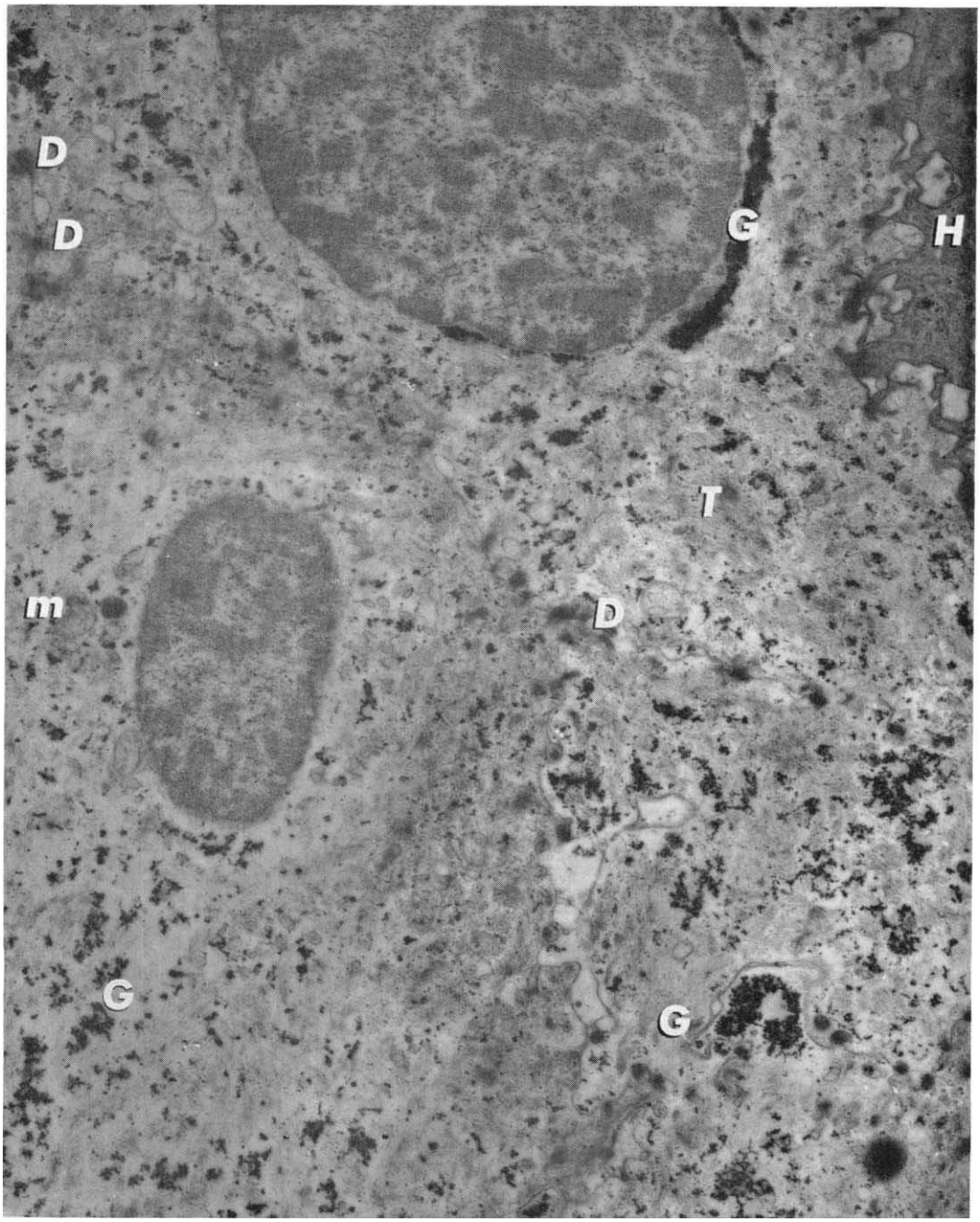

FIG. 2. *Glycogen particles in upper epidermis, 13 weeks old embryo.* Glycogen particles are seen in the cytoplasm (G). D: desmosomes. H: horny cell. m: mitochondria. T: tonofilaments. (× 11,850.)

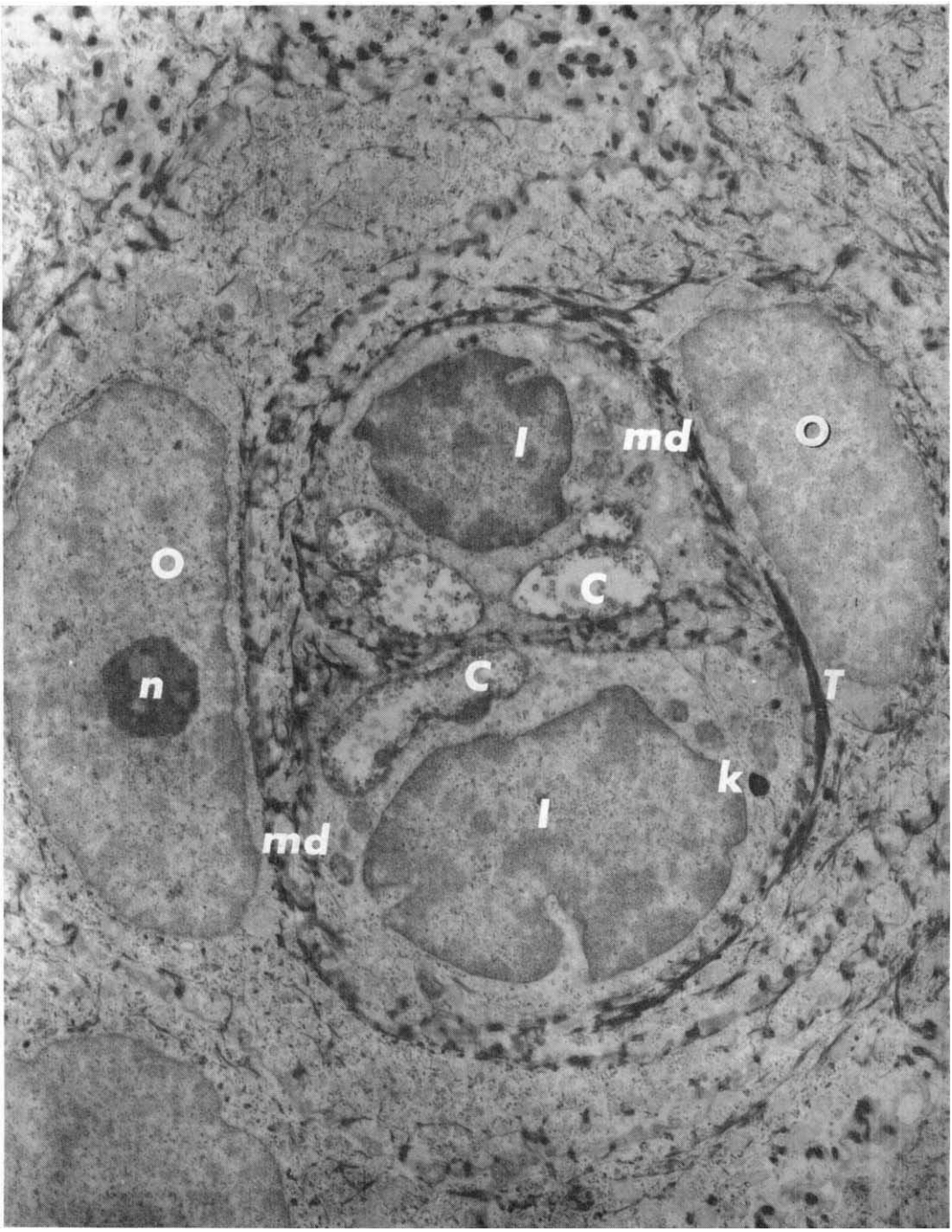

FIG. 3. *Formation of lumen in inner cells, mid-squamous layer, 16 weeks old embryo.* Cavities (C) are being formed within two apposing inner cells (I) by the pinching off of part of the cytoplasm. Pinched-off cytoplasm is seen as small vesicles in these cavities. Between the inner cells lie the intact plasma membranes. Two outer cells (O) flank the inner cells. Prominent bundles of tonofibrils (T) are arranged along the inner, concave side of the outer cells. k: keratohyaline granules. md: multivesicular dense bodies. n: nucleolus of the outer cell. (× 7,900.)

lumen formation, intracytoplasmic cavities were formed within the cells of the inner cells by a resolution and pinching-off of cytoplasm (Fig. 3). The pinched off cytoplasm appeared as multiple, membrane-lined "vesicles" within the newly formed cavities. Having enlarged and coalesced with one another (Fig. 3), these cavities broke through the cell membrane and united with similarly produced cavities of adjoining inner cells to form a lumen that was located extracellularly (Fig. 4). Ultimately a patent lumen extended along the entire length of the duct by coalescence of the lumina thus formed in the inner cells at different levels of the epidermis (Fig. 5). The luminal border, then, was lined by the same pinching villi as lined the cavities of the inner cells.

Gradually, however, numerous microvilli formed at the luminal border of the inner cells. Tonofilaments were visible within the microvilli at the lower level of the epidermis. Immediately external to the microvilli was the terminal web. Peripheral to the terminal web, there was an interwoven network of tonofilaments (periluminal filamentous zone) (Fig. 6). The inner cells contained a few homogeneously dense keratohyaline granules (Fig. 7) which increased in number in the mid- to upper squamous layers (Figs. 3, 7). The inner cells contained an abundance of multivesicular dense bodies (Fig. 6).

The presence of multivesicular dense bodies was the most reliable identifying feature of the inner cell, enabling the inner cells to be recognized even before any intracellular cavities had formed. These multivesicular dense bodies consisted of an aggregation of small vesicles and a dense substance (probably protein), around which an electron-dense double membrane subsequently formed. Some of these small vesicles showed cristae (Fig. 6).

In the stratum granulosum, where the inner cells contained many large keratohyaline granules, the lumen was formed by the same mechanism described above, *i.e.* through the formation and subsequent merging of multiple cavities (Fig. 8). At the level of the upper squamous and granular layers the microvilli lining the sweat duct became homogeneously dense (Fig. 7). Fewer multivesicular dense bodies were found in the upper epidermis than in the lower epidermis. Endoplasmic reticulum and Golgi apparati were more abundant in the inner cells than in the outer cells.

Outer cells.—The cells of the outer cylinder were elongated and curved so that they embraced the inner cylinder (Figs. 3, 4). The nuclei were also elongated. Bundles of tonofibrils forming a dense ring often lay in the cytoplasm along their inner concave borders (Fig. 3). These fibrils, together with the periluminal filamentous zone of the inner cells seemed to serve as a supporting ring to maintain the patency of the lumen. Aggregates of glycogen granules were present in the cells of the outer cylinder at the lower level of the epidermis, but the amount was usually far less than in the surrounding squamous cells (Fig. 4). The amount of glycogen in the surrounding cells, however, fluctuated from specimen to specimen. Irregularly shaped keratohyaline granules were already present in moderate number in the outer cells at the level of the mid-squamous layer, in contrast to the inner cells and the surrounding cells of the epidermis which contained few keratohyaline granules at this level. The keratohyaline granules became more prominent at the level of the upper epidermis. Those present in the outer cells were polygonal or stellate in shape and attached by tufts of tonofilaments. They were morphologically identical with the keratohyaline granules found in the granular cells of the epidermis.

Small round granules of medium electron density were found in great numbers in the cells with keratohyaline granules or in the cells subjacent to them (Fig. 7). These granules ranged in size from 100 mμ to 200 mμ, demonstrated internal dense areas or cristae, and were enveloped with a double membrane. They resembled mitochondria, but were much smaller in size. Morphologically they appeared to be identical with the *"membrane-coating granules"* of Matoltsy and Parakkal (3). As they described, these granules tended to gather close to the plasma membranes. Although there was no direct evidence that these granules were emptying their contents into the intercellular spaces as Matoltsy and Parakkal (3) found, the intercellular spaces between or near the cells which carried these granules were often filled with an amorphous material of medium electron density (Fig. 7). Also, the plasma membranes of the inner and outer cells thick-

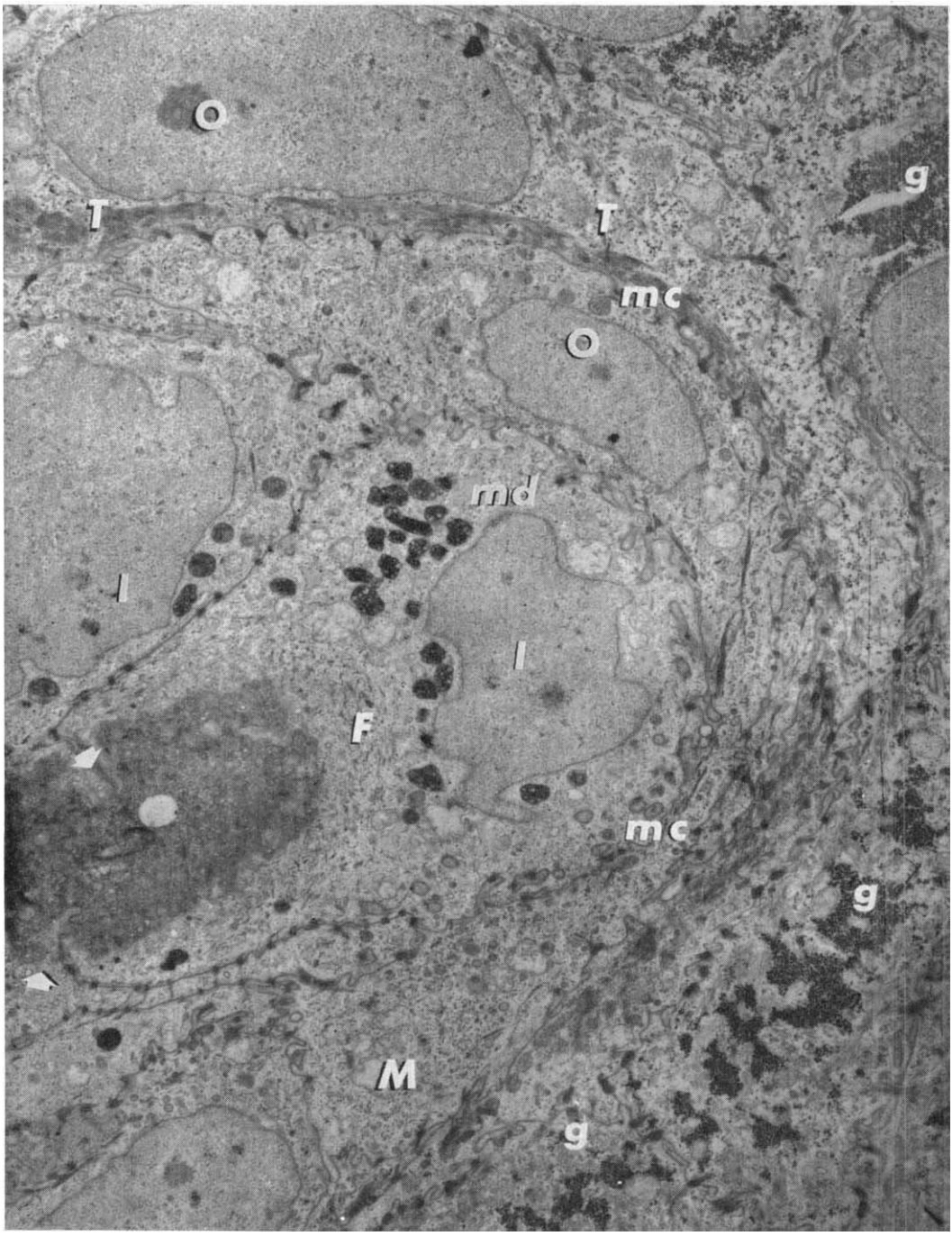

FIG. 4. *Coalescence of cavities between two inner cells, basal layer, 16 weeks old embryo.* Enlarged cavities in two apposing inner cells (I) are now in communication with each other (arrows). Pinched-off villi and small vesicles fill the lumen. Numerous multivesicular dense bodies (md) are present in the inner cells (I) but not in the outer cells (O). Squamous cells surrounding the outer cells contain an abundant amount of glycogen particles (g). F: periluminal filamentous zone. M: mitochondria. mc: membrane-coating granules. T: tonofibrillar band along the inner, concave border of the outer cell. (× 6,545.)

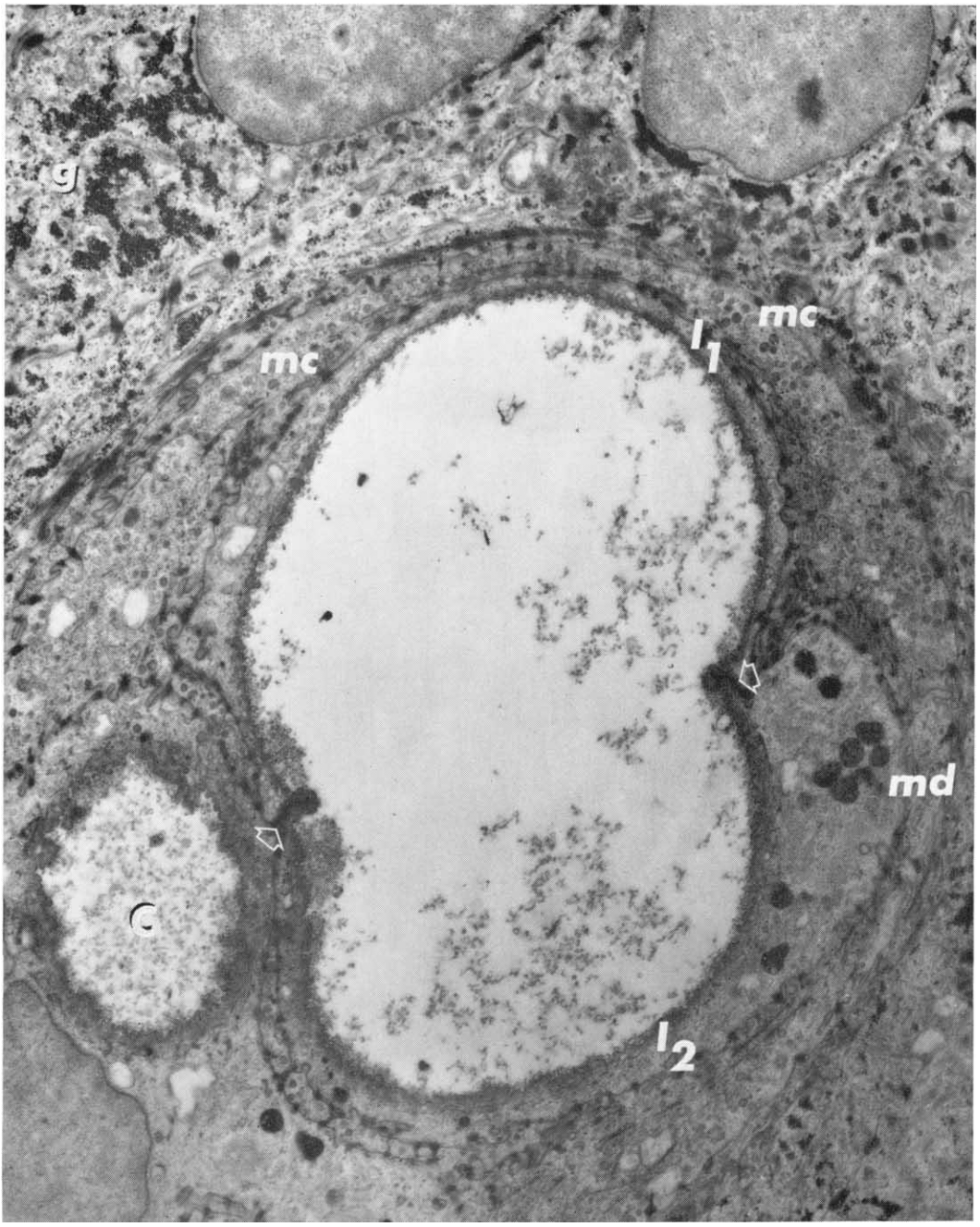

FIG. 5. *Patent duct, 16 weeks old embryo, mid-squamous layer.* The lumen still contains numerous small vesicles but is now patent. Lumina in two apposing inner cells (I_1 and I_2) have become enlarged to occupy almost the entire space of the cells. Vestiges of former cell membranes which separated two inner cells can be observed as tiny projections sticking out from both sides at an isthmus (arrows). Intracellular cavities (C) are formed in an inner cell subjacent to the other two. g: glycogen particles in squamous cells. mc: membrane-coating granules. md: multivesicular dense bodies. (× 6,545.)

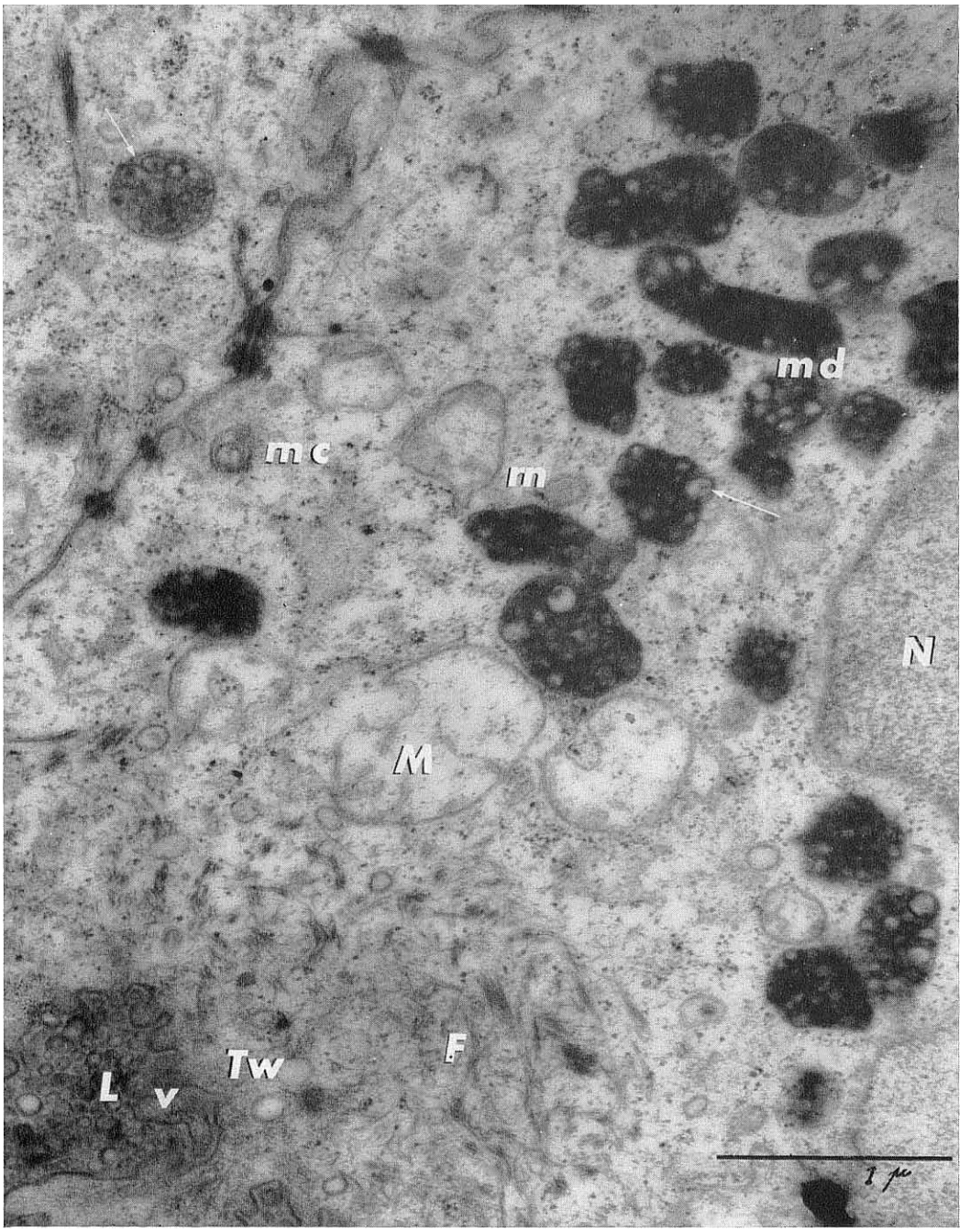

FIG. 6. *Multivesicular dense bodies, basal layer, 16 weeks old embryo.* This cell contains many moderately electron-dense membrane-coating granules (mc), some of which show a double membrane and internal structure. Multivesicular dense bodies (md) contain vesicles which show cristae (arrow). The luminal border of the cell bears microvilli (v). Pinched-off villi fill the lumen (L). F: periluminal filamentous zone. M: mitochondria. N: nucleus of inner cell. Tw: terminal web. (× 32,340.)

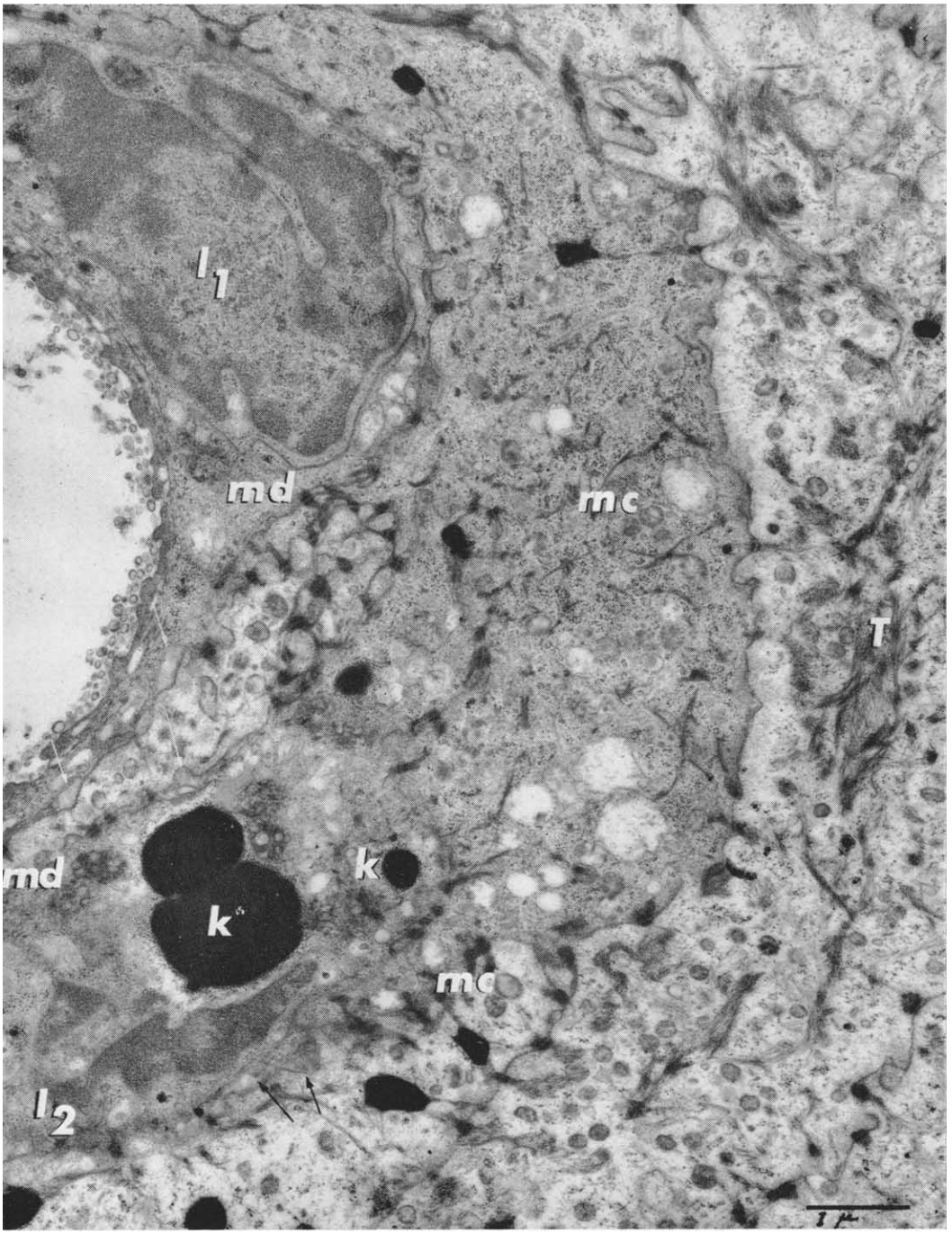

FIG. 7. *Inner cells in the upper squamous layer, 16 weeks old embryo.* Two inner cells are shown (I_1, I_2). Keratohyaline granules (k) appear in the lower inner cell which does not surround the lumen in this section, but multivesicular dense bodies (md) in the cytoplasm testify to its being an inner cell. The microvilli on the luminal border appear homogeneously dense. Arrows: homogeneous intercellular material. mc: membrane-coating granules. T: tonofibrillar band along the inner, concave border of the outer cell. (× 21,000.)

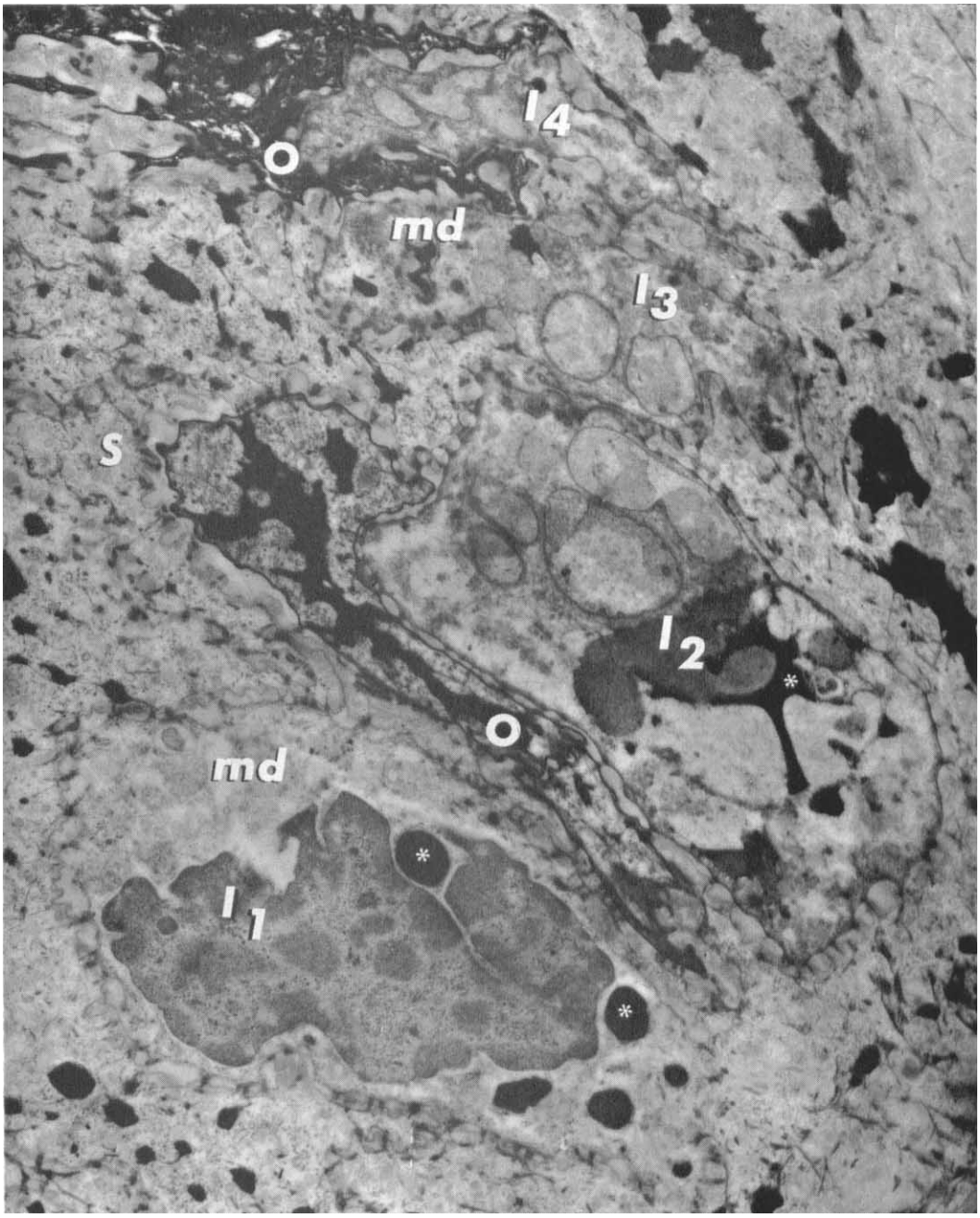

FIG. 8. *Inner cells in the upper squamous layer and granular layer, 16 weeks old embryo.* Four inner cells with keratohyaline granules and thickened plasma membranes are cut in one plane; one cell in the upper squamous layer (I_1) and three cells in the granular layer (I_2, I_3, I_4). The outer cells (O) surrounding I_2 and I_4 show an advanced stage of keratinization, while the surrounding squamous cells (S) show less degree of keratinization. Multiple intracytoplasmic cavities with numerous pinched-off vesicles are revealed in I_2, I_3 and I_4, while no cavity is cut in this section in I_1. Many multivesicular dense bodies (md) are found in each inner cell. *: keratohyaline granules. (× 16,200.)

ened as they gradually lost these granules undergoing keratinization in the upper epidermis.

Cross-sections of the sweat ducts showed that two, and occasionally three, inner cells and three to four outer cells surrounded the lumen. The inner and outer cells were closely united with each other by desmosomes and the outer cells with the surrounding cells of the epidermis.

In embryos 22 weeks old (18 cm long), maturation had progressed to the stage where the intraepidermal eccrine ducts almost resembled those of the adult (4, 5). The outer cylinder was now composed of two to three layers of cells which gradually merged in the surrounding epidermal cells. The periluminal filamentous zone of the inner cell became more prominent.

At the level of the stratum granulosum, the cells of the inner layer, though flattened and partially keratinized, still contained numerous vesicles in various-sized luminal projections (Fig. 9). The outer cells, however, were completely keratinized. In addition, the keratinized outer cells were surrounded by several additional layers of keratinized epidermal cells (Fig. 9). The development of a periluminal filamentous zone and the earlier keratinization of the ductal and periductal cells appeared to strengthen the ductal structure and contribute to the luminal patency.

In the stratum corneum the inner cells shed into the lumen without undergoing complete keratinization through the loss of their desmosomal attachments to the surrounding outer cells (Fig. 10). The lumen contained numerous small vesicles (Fig. 10), probably carried up from the lower portion of the duct. As the inner cells were being shed, the completely keratinized cells of the outer cylinders came to line the lumen; then in turn they too were shed (Fig. 10).

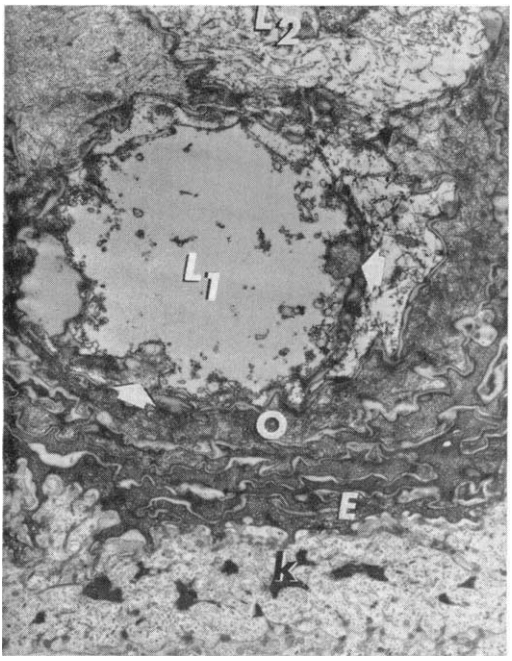

FIG. 9. *Duct in the granular layer, 22 weeks old embryo.* The inner cells show various shaped balloon-like projections (arrows) of the luminal border which contain numerous vesicles. The rest of the cytoplasm is partially keratinized. The outer cells (O) and several surrounding epidermal cells (E) are completely keratinized. The ductal lumen (L_1) contains numerous vesicles. A portion of the lumen in the horny layer (L_2) is seen. k: keratohyaline granules of surrounding granular cells. (× 17,000.)

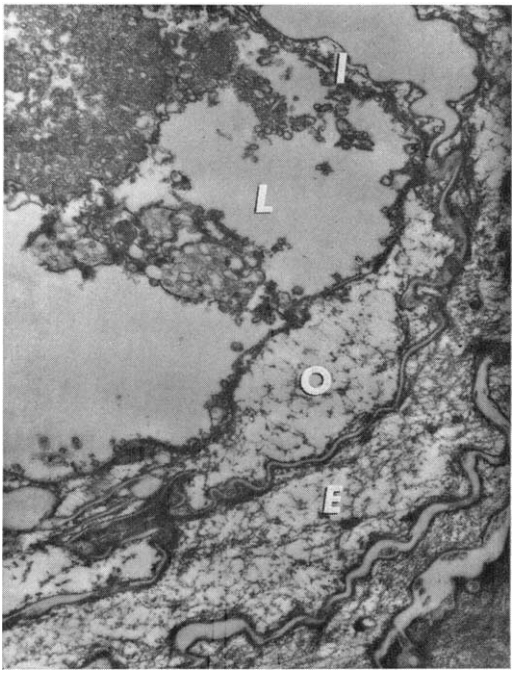

FIG. 10. *Duct in the horny layer, 22 weeks old embryo.* The inner cell (I) is shedding into the lumen (L) which contains numerous vesicles pushed up from the lower epidermis. The surrounding outer cell (O) and epidermal cells (E) have disintegrated and are also being shed into the lumen. (× 22,000.)

DISCUSSION

Inner cells and multivesicular dense bodies.—The inner cells contribute part of their own cytoplasm to the newly forming duct by excreting segments of themselves. Thus, the formation of the lumen is initiated intracellularly rather than extracellularly.

It is interesting that the human embryo in the second trimester is already provided with adult eccrine sweat duct structures (4–7) such as the keratinizing ductal wall, luminal microvilli and periluminal filamentous zone and prepared for extra-uterine life. The multivesicular dense bodies described in this report have also been observed in adult intraepidermal eccrine sweat ducts (7), though in much smaller amounts. Multivesicular dense bodies containing acid phosphatase activity have been described in other periductal cells, *i.e.*, in the pericanalicular cells of the bile duct (8) and in the cells of the proximal convoluted tubules of the mouse kidney (9). This type of multivesicular dense body has also been described in the intestinal epithelium of the bullfrog during metamorphosis (10). It seems most likely that the multivesicular dense bodies observed in the inner cells represent periductal lysosomes playing an important role in the autolytic formation of the intraepidermal eccrine sweat duct.

Membrane-Coating Granules. Membrane-coating granules, as described by Matoltsy and Parakkal (3), are round or oval in shape, range from 100 mμ to 500 mμ in size, are encapsuled in smooth walled membranes, and have an internal structure consisting of parallel membranes. Thus, the small round granules found in the ductal wall cells in this study seem to be identical with these granules. The membrane-coating granules, according to Matoltsy and Parakkal (3), fuse with the plasma membranes, empty their contents (probably polysaccharides) into the intercellular spaces in the upper strata of the keratinizing epithelium and coat the plasma membranes from outside. In this study an amorphous material was seen in the intercellular spaces, and a certain thickening of the plasma membranes was observed in the keratinizing ductal cells. These findings are compatible with the fact that the ductal wall cells of embryos are keratinizing epithelium.

SUMMARY

1. The development of the intraepidermal eccrine sweat duct was studied with the electron microscope using specimens from human embryos 12–22 weeks old.

2. Anlagen of eccrine sweat glands were first seen in embryos 12–13 weeks old. Intraepidermal sweat ducts first appeared in embryos 14–15 weeks old. Cells destined to line the lumen were recognizable, however, before the lumen formed since the inner cells contained multivesicular dense bodies, which probably represent pericanalicular lysosomes.

3. In the process of formation of the lumen, intracellular cavities formed within the inner cells. These cavities enlarged, coalesced and broke through the cell membranes. Through the uniting of similarly produced cavities of adjoining inner cells patent extracellular lumens were formed.

4. The outer cells began to keratinize at the level of the mid-squamous layer, while the inner cells and the surrounding epidermal cells showed few keratohyaline granules at this level. In the stratum corneum, however, the inner cells underwent partial keratinization and shed into the lumen. The innermost layer of the duct was then lined by the surrounding outer cells which were completely keratinized and shed into the lumen.

5. In young embryos (12–13 weeks old) glycogen particles were present in the intercellular spaces of the eccrine sweat gland anlagen as well as between the cells of the stratum germinativum. In the stratum intermedium the glycogen particles appeared in the cytoplasm; and, as the upper strata of the epidermis was approached, the amount of intracellular glycogen increased.

6. The membrane-coating granules of Matoltsy and Parakkal were present in abundance in the keratinizing ductal wall cells. An amorphous substance of medium electron density, probably derived from these granules, was present in the intercellular spaces.

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